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# In Vitro Laser Welding of Amniotic Membranes

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***Objective:*** **To test in vitro the feasibility of welding amniotic membranes using Nd:YAG laser energy.**
***Study design:*** **Fresh fetal membranes from term pregnancies were washed and cut into 1 $cm^2$ pieces. Pooled cryoprecipitate (CPT), 50% albumin (Alb), or polytetrafluoroethilene (e-PTFE) were used as solder medium. The optimal settings of the laser were determined. Results were assessed quantitatively and semi-quantitatively using Pearson Chi-square analysis.**
***Results:*** **Laser welding of amniotic membranes was successful in 82.6% of experiments with e-PTFE and in 10.7% of experiments with CPT ($P < 0.001$). The strength of the welding was also significantly better with e-PTFE ($P < 0.001$). Optimal results were obtained using 1–7 Watts and 0.1–1 seconds. Laser welding was unsuccessful in 100% of experiments with Alb.**
***Conclusions:*** **Laser welding of fetal membranes can be accomplished with e-PTFE and to a lesser degree with the CPT using Nd:YAG energy under low wattage-high exposure settings. Further studies are underway to test other grafting or soldering materials. Lasers Surg. Med. 24:315–318, 1999.**
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**Key words: amnion; Nd:YAG; premature rupture of membranes; tissue welding**

## INTRODUCTION

Preterm premature rupture of membranes (PPROM) accounts for 30–40% of all preterm deliveries in the United States [1], 25% occurring before the 26th week of gestation. Spontaneous healing of the amniotic membranes is infrequent. Patients continue to leak fluid for the rest of the pregnancy increasing the risk of perinatal complications such as infection, respiratory insufficiency, intraventricular hemorrhage, pulmonary hypoplasia, cerebral palsy, and death [2,3].

There is currently no treatment capable of sealing the membranes in patients with PPROM. To date, only two reports document effective treatment of PPROM with membrane sealing. Quintero et al., successfully used transabdominal intraamniotic injection of platelets and cryoprecipitate in a patient who had ruptured membranes after endoscopic fetal surgery [4]. Sener placed maternal blood in the chorionic cavity in a patient with iatrogenic PPROM [5]. However, these and other techniques have not been successful in sealing the membranes in spontaneous PPROM [6,7].

Laser energy is known to weld tissues by changing the triple helix structure of collagen and yielding noncovalent bonds [8]. This technique has been used as a substitute or reinforcement of traditional suture closures in urology, vascular anastomoses, and pleural and cerebrospinal fluid fistulas [9–11]. There is no experience with this technique in obstetrics. The purpose of this study was to test the feasibility of welding amniotic membranes in vitro using Nd:YAG laser energy.

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**TABLE 1. Percent Success Rate**

| Medium | No. of attempts | Total welded | % |
|---|---|---|---|
| e-PTFE | 144 | 119 | 82.6% |
| CPT | 328 | 35 | 10.7% |
| Albumin | 76 | 0 | 0.0% |

## MATERIALS AND METHODS

Fresh fetal amniotic membranes were obtained from uncomplicated term pregnancies after vaginal or abdominal delivery. Membranes were thoroughly washed with 0.9 normal saline solution to remove blood clots and were cut into 1 $cm^2$ pieces. Pooled cryoprecipitate (CPT) with a fibrinogen concentration of 1,600–1,700 mg/dl and 50% human albumin (Alb) were used as solder. Polytetrafluoroethilene (e-PTFE) material (1 $cm^2$, 0.3–0.4 mm in thickness (Gore-Tex Co., Gore and Associates, Flagstaff, AZ) was also tested as a graft placed over the membranes. A Nd:YAG laser (wave length 1,064 nm, Surgical Laser Technologies Inc., Montgomery, PA) in pulse mode served as the energy source. A noncontact fiber (Surgical Laser Technologies Inc., Montgomery, PA) was used for both the CPT and Alb experiments, and a contact fiber was utilized for the e-PTFE experiments. The pulse duration and wattage were systematically varied using 15 or 30 seconds of exposure to determine the optimal settings. The resistance of the welded tissue to separate under manual traction was assessed subjectively (0 = negative, 1 = moderate, 2 = strong) using Pearson chi-square analysis. The thermal effect on the tissues was assessed histologically.

For each experiment, two membrane pieces were placed on a ceramic plate with the amnion layer face up and with the edges of two segments placed side-by-side, touching edges, with no measurable distance between them. CPT or Alb was placed between the pieces with a hypodermic needle. Five to 60 Watts was used for the CPT experiments and 1–15 Watts for the Alb experiments, as tissue degeneration was noted beyond this level. The pulse duration varied between 0.1–10 seconds. For the e-PTFE experiments, the graft was placed over the amnion layer of the two pieces of amniotic membranes and 1–10 Watts were used with a pulse duration of 0.1–1 seconds. Experiments were done in duplicate.

## RESULTS

Results are summarized in Table 1. Laser welding was successful in 82.6% of sets with e-PTFE and in 10.7% of experiments with CPT ($P < 0.001$). Laser welding was unsuccessful in 100% of experiments with Alb. The strength of the welding was significantly better with e-PTFE (50.4% of e-PTFE vs. 37.1% of CPT, $P < 0.001$) (Table 2).

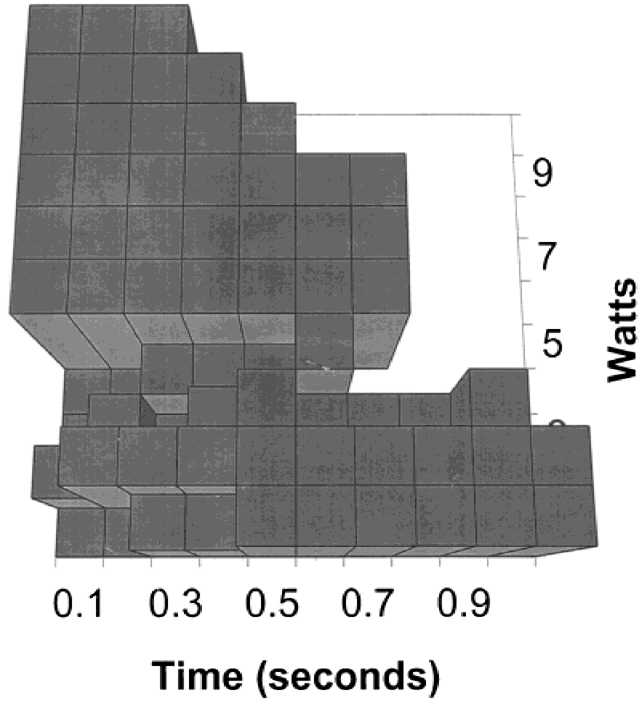


Fig. 1. Frequency and quality of welding of e-PTFE and amnion in relationship to wattage and exposure time. Two areas of optimal settings can be identified: low-wattage/high exposure vs. high-wattage/low exposure, represented by the taller bars. Lower bars represent successful welding but of lower strength. The clear areas represent unsuccessful welding.

Figure 1 shows the distribution of positive results with e-PTFE relative to different Nd:YAG laser settings. Two different combinations of exposure time and wattage were found to yield the best results, namely low wattage (1–2 Watts) for 0.5–1 seconds vs. 5–10 Watts for less than 0.3 seconds. Optimal results were obtained using 1–7 Watts of laser energy and 0.1–1 seconds of exposure time with e-PTFE, and 5–15 Watts of laser energy and 15 seconds of exposure time with CPT. Alterations in the color and shrinkage in the amniotic membranes was seen with more than 15 Watts with increased exposure time. Using lower setting, minimal denudation of the amniotic epithelium, and no changes in the chorion layer were observed (Fig. 2).

## DISCUSSION

Our study shows that, given a particular set of conditions, laser welding of human amniotic

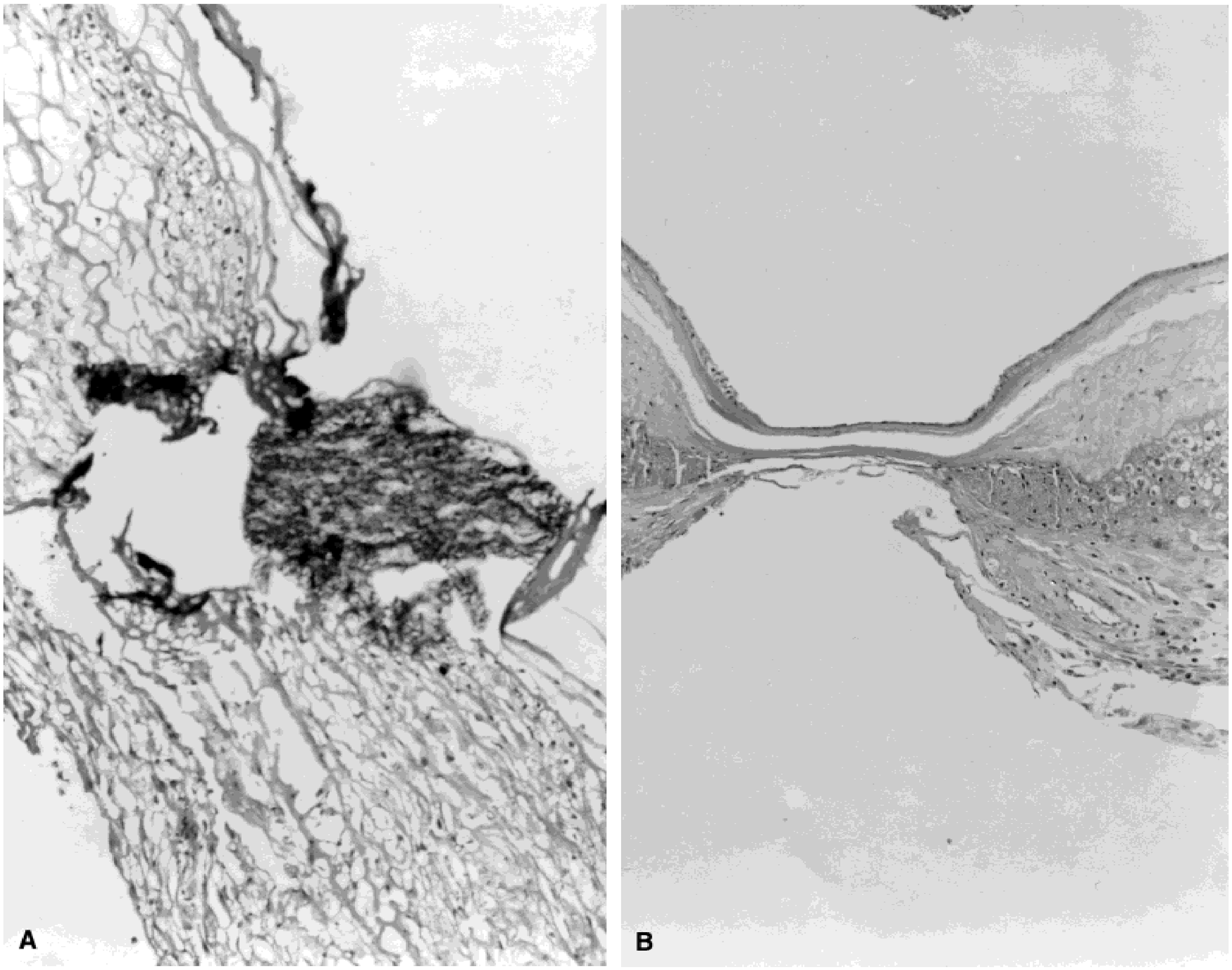


Fig. 2. Surgical pathology specimen showing laser welding of amniotic membranes. **A:** Welding of e-PTFE material, showing attachment of the material to the amnion. **B:** Histologic section showing site of welding, with minimal denudation of the amnion; the welded tissues are not shown in this slide, as they separate artificially during specimen preparation.

**TABLE 2. Strength of the Welded Tissues**

| Medium | Total | Moderate N (%) | Strong N (%) |
|---|---|---|---|
| e-PTFE | 119 | 59 (49.6) | 60 (50.4) |
| CPT | 35 | 22 (62.9) | 13 (37.1) |

membranes can be accomplished. The emphasis of this preliminary work was to define those conditions necessary for the welding to take place.

Laser welds tissues by producing noncovalent bonds from denatured collagen [12]. The temperature necessary for these changes to take place is in the range of 60–80°C. Because the ultimate tissue temperature generated by the laser is relatively unpredictable due to variations in local factors including tissue absorption and thermal conductivity, there is a narrow margin between a successful and unsuccessful weld. Too little laser energy results in a weak or nonexistent bond, whereas excessive energy results in drying and shrinkage of the tissue proteins, reducing weld flexibility and strength [12]. Clinically, the endpoint can be observed if there is a color or surface change in the tissues, but these grossly visible changes are well past the ideal endpoint. Controlled heating is facilitated by pulsing the laser light. As shown in this study, the success of laser welding depends on a subtle balance of the two variables of power and exposure time.

Albumin in a 50% concentration and CPT have been used as solder media to increase the strength of the weld [13,14]. We were not able to corroborate this experience in 76 sets of experiments using 50% Alb because the energy required to produce appropriate changes in the Alb was excessive for the amnion. CPT produced reliable tissue welding in only 10% of the sets, 62.9% of which were of moderate-strong strength.

The best laser welding results were obtained with e-PTFE. This material has been used successfully in the repair of bronchopleural fistulas [15]. Two different settings of power and exposure time yielded the best results: 1–2 Watts for 0.5–1 seconds (low wattage/high exposure) vs. 5–10 Watts for 0.3 seconds or less (high wattage/low exposure). Of these two combinations, optimal results were obtained using 1–7 Watts of laser energy and 0.1–1 seconds of exposure time with e-PTFE. In the case of CPT, 5–15 Watts of laser energy and 15 seconds of exposure time yielded the best results.

We believe that laser welding of fetal membranes can be accomplished with e-PTFE and CPT under ideal Nd:YAG settings of power and exposure time. Given the avascular nature of the amniotic membranes and the slow repair rate of amnion relative to other cell lines [16], we believe that e-PTFE could be used as a patch to support the repairing process of the amniotic membranes. A minimally invasive or transcervical technique would be required to deliver and weld the graft material to the site of membrane rupture. The surgical technique and the use of other grafting or soldering materials is currently being pursued at our laboratory. Laser welding of amniotic membranes in patients with spontaneous PPROM would have important medical and economical implications in obstetrics.

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